

SEQUENCE LISTING

RECEIVED
MAY 28 2002
TECH CENTER 1600/2900

<110> DING, Jeak Ling
TAN, Nguan Soon
HO, Bow
LAM, Toong Jin

<120> ISOLATED NUCLEIC ACIDS ENCODING A SECRETORY SIGNAL FOR EXPRESSION AND
SECRETION OF HETEROLOGOUS RECOMBINANT PROTEINS

<130> 1781-0178P

<140> US 09/426,776

<141> 1999-10-26

<160> 22

<170> PatentIn version 3.0

<210> 1

<211> 29

<212> DNA

<213> Artificial

<220>

<223> Chloramphenicol acetyltransferase (CAT) gene forward primer derived
from bacteria

<400> 1

gaagatctgc tggagaaaaa aatcactgg

29

<210> 2

<211> 29

<212> DNA

<213> Artificial

<220>

<223> Chloramphenicol acetyltransferase (CAT) gene forward primer derived
from bacteria

<400> 2

gcacggccg tgccttaaaa aaattacgc

29

<210> 3

<211> 21

<212> DNA

<213> Artificial

<220>

<223> OaVtgExon2 reverse primer derived from Oreochromis aureus vitellogenin
gene exon 2

<400> 3

ccaagttgga ctggtccccc a

21

<210> 4



B2

<211> 19
 <212> DNA
 <213> Artificial

 <220>
 <223> EGFP reverse primer derived from Aequoria victoria green fluorescent protein

 <400> 4
 ccctcgccgg acacgctga 19

 <210> 5
 <211> 29
 <212> DNA
 <213> Artificial

 <220>
 <223> B-lactamase forward primer derived from bacteria

 <400> 5
 ccgggatcca gaaacgctgg tgaaagtaa 29

 <210> 6
 <211> 29
 <212> DNA
 <213> Artificial

 <220>
 <223> B-lactamase reverse primer derived from bacteria

 <400> 6
 gcggccgtta ccaatgctta atcagtgag 29

 <210> 7
 <211> 29
 <212> DNA
 <213> Artificial

 <220>
 <223> Forward primer from BspSS

 <400> 7
 gggcatgag ggtgcttgta ctagctctt 29

 <210> 8
 <211> 30
 <212> DNA
 <213> Artificial

 <220>
 <223> BamGal forward primer with BamHI restriction site and some beta-galactosidase sequence derived from bacteria

 <400> 8
 ccatggatcc cgtgatttcg ttgccggtct 30

<210> 9
 <211> 26
 <212> DNA
 <213> Artificial

 <220>
 <223> EagGal reverse primer with EagI restriction site

 <400> 9
 gcgacggccg ggcagacatg gcctgc 26

 <210> 10
 <211> 21
 <212> PRT
 <213> Oreochromis aureus

 <400> 10

 Met Arg Val Leu Val Leu Ala Leu Ala Val Ala Leu Ala Val Gly Asp
 1 5 10 15

 Gln Ser Asn Leu Gly
 20

 <210> 11
 <211> 80
 <212> DNA
 <213> Oreochromis aureus

 <220>
 <221> CDS
 <222> (18)..(80)
 <223> Nucleotides 18-80 encode for SEQ ID NO: 10

 <400> 11
 attcacatcc accagcc atg agg gtg ctt gta cta gct ctt gct gtg gct 50
 Met Arg Val Leu Val Leu Ala Leu Ala Val Ala
 1 5 10

 ctc gca gtg ggg gac cag tcc aac ttg ggg 80
 Leu Ala Val Gly Asp Gln Ser Asn Leu Gly
 15 20

 <210> 12
 <211> 204
 <212> DNA
 <213> Artificial

 <220>
 <223> Junction of Vtgss (derived from Oreochromis aureus) and CrFCES
 (Carcinoscorpius rotundicauda ES - EcoRI-SalI flanking fragment of
 Factor C) determined by sequencing using the Ac5 forward primer and
 pcDNA3.1/BGH reverse primer

 <400> 12

gtggaaattct gcagatgcta ccggactcag atcaattcac atccaccagc catgagggtg 60
 cttgtactag ctcttgctgt ggctctcgca gtgggggacc agtccaactt gggggatcta 120
 ggcttggtg atgaaacgag gttcgagtgt aagtgtggcg atccaggcta tgtgttcaac 180
 attccagtga aacaatgtac atac 204

<210> 13
 <211> 51
 <212> PRT
 <213> Artificial

<220>
 <223> VtgCrFCES protein - Vtg derived from Oreochromis aureus and CrFCES
 derived from Carinoscorpius rotundicauda ES - EcoRI-SalI flanking
 fragment of Factor C

<400> 13

Met Arg Val Leu Val Leu Ala Leu Ala Val Ala Leu Ala Val Gly Asp
 1 5 10 15
 Gln Ser Asn Leu Gly Asp Leu Gly Leu Cys Asp Glu Thr Arg Phe Glu
 20 25 30
 Cys Lys Cys Gly Asp Pro Gly Tyr Val Phe Asn Ile Pro Val Lys Gln
 35 40 45
 Cys Tyr Phe
 50

<210> 14
 <211> 152
 <212> DNA
 <213> Artificial

<220>
 <223> Part of the Vtgss-CAT (Vtgss from Oreochromis aureus - CAT of bacterial
 origin) fusion in the pBSVtgCAT vector

<400> 14
 atcgataagc ttgatgctac cggactcaga tcaattcaca tccaccagcc atgagggtgc 60
 ttgtactagc tcttgctgtg gctctcgag tgggggacca gtccaacttg ggggatctgc 120
 tggagaaaaa aatcactgga tataccaccg tt 152

<210> 15
 <211> 59
 <212> DNA
 <213> Artificial

<220>
 <223> Part of the Vtgss-CAT (Vtgss from Oreochromis aureus - CAT of bacterial
 origin) fusion in the pBSVtgCAT vector

<400> 15
 ggcggggcgt aattttttta aggcacggcc gatgcgacgg tatcgataac ttgatatcg 59

<210> 16
 <211> 34
 <212> PRT

<213> Artificial

<220>

<223> Part of the Vtgss-CAT (Vtgss from *Oreochromis aureus* - CAT of bacterial origin) fusion in the pBSVtgCAT vector

<400> 16

Met Arg Val Leu Val Leu Ala Leu Ala Val Ala Leu Ala Val Gly Asp
1 5 10 15
Gln Ser Asn Leu Gly Asp Leu Leu Gln Lys Lys Val Thr Gly Trp Thr
20 25 30
Thr Val

<210> 17

<211> 3

<212> PRT

<213> Artificial

<220>

<223> Part of the Vtgss-CAT (Vtgss from *Oreochromis aureus* - CAT of bacterial origin) fusion in the pBSVtgCAT vector

<400> 17

Gly Gly Ala
1

<210> 18

<211> 66

<212> DNA

<213> Artificial

<220>

<223> Part of the nucleotide sequence adjoining Vtgss (derived from *Oreochromis aureus*) and CAT (derived from bacteria) in the vector psp-VtgCAT

<400> 18

ggcggggcgt aattttttta aggcacggcc gatgcgacgg tatcgatatt gttacaacac 60
cccaac 66

<210> 19

<211> 155

<212> DNA

<213> Artificial

<220>

<223> Nucleotide sequence of the Vtg-EGFP (Vtg derived from *Oreochromis aureus* - EGFP derived from *Aequoria victoria*) fusion in the vector pVtgEGFP

<400> 19

gctagcgcta ccggactcag atcaattcac atccaccagc catgaggggtg cttgtactag 60
ctcttgctgt ggctctcgca gtgggggacc agtccaactt gggggatcca ccggtcgcca 120
ccatggtgag caagggcgtg gtgcagaact ccggg 155

<210> 20
<211> 38
<212> PRT
<213> Artificial

<220>
<223> Amino acid sequence of the Vtg-EGFP (Vtg derived from *Oreochromis aureus* - EGFP derived from *Aequoria victoria*) fusion in the vector pVtgEGFP

<400> 20

Met Arg Val Leu Val Leu Ala Leu Ala Val Ala Leu Ala Val Gly Asp
1 5 10 15
Gln Ser Asn Leu Gly Asp Pro Pro Val Ala Thr Met Val Ser Lys Gly
20 25 30
Val Val Gln Asn Ser Gly
35

<210> 21
<211> 204
<212> DNA
<213> Artificial

<220>
<223> Nucleotide sequence at the junction of Vtgss (derived from *Oreochromis aureus*) and B-lactamase (derived from bacteria) in pBADVtgblactKana

B2 <400> 21
ctctactgtt tctccataacc cgtttttttg ggctaacagg aggaattaac catgaggggtg 60
cttgtagtag ctcttgctgt ggctctcgca gtgggggacc agtccaactt gggggatcca 120
gaaacgctgg tgaaagtaaa agatgctgaa gatcagttgg gtgcacgagt gggttacatc 180
gaactggatc tcaacagcgg taag 204

<210> 22
<211> 51
<212> PRT
<213> Artificial

<220>
<223> Amino acid sequence at the junction of Vtgss (derived from *Oreochromis aureus*) and B-lactamase (derived from bacteria) in pBADVtgblactKana

<400> 22

Met Arg Val Leu Val Leu Ala Leu Ala Val Ala Leu Ala Val Gly Asp
1 5 10 15
Gln Ser Asn Leu Gly Asp Pro Glu Thr Leu Val Lys Val Lys Asp Ala
20 25 30
Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp Leu Asn
35 40 45
Ser Gly Lys
50